SHORTER NOTES

A GERMINATION METHOD FOR ISOËTES. — Engelmann (Trans. Acad. Sci. St. Louis 4:359–390. 1886), Campbell (Ann. Bot. 5:231–258, *t. XV–XVII*. 1891), La Motte (Amer. J. Bot. 20:217–233. 1933; Ann. Bot. n.s. 1:695–715. 1937), and Boom (Amer. Fern J. 70:1–4. 1980) all described a similar procedure for germinating *Isoëtes* spores in order to obtain endosporic megagametophytes. Their methods, however, allow the introduction of soil on the spore wall, archegonial pad, and among the rhizoids. This makes necessary the tedious separation of all mud and grit from the plant material before embedding and sectioning if torn ribbons and nicks in the microtome knife are to be avoided, according to Johansen (Plant Microtechnique, 1940). Recently I was successful in germinating megaspores of *I. engelmannii* A. Br. on an inorganic nutrient medium, which totally avoids the problem of soil on the specimens.

Material of *I. engelmannii* was collected on 5 Sept 1981, at a time when the spores were nearly mature. All plants were from the lake at Hanging Rock State Park, one of the most easterly mountain areas in North Carolina, located 32 miles north of Winston-Salem on roads NC-89 and NC-66. Mature, fertile plants along with adhering mud were removed from the bottom of the lake with as little disturbance as possible and were brought to the Duke University greenhouse. Inact plants were transplanted for future studies. Sporophylls broken off in moving the plants were used in culture studies.

The nutrient medium was that of Dr. B. C. Parker, Washington University, St. Louis, Missouri, which was used by Klekowski (J. Linn. Soc. London 62:361–377. 1969) for culturing gametophytes of the Blechnaceae. This medium was slightly modified by adding 0.30 g per liter of Mycostatin (=Nystatin, available from SIGMA) and 15 g (rather than 10 g) of agar per liter of nutrient solution. All but the very base of the sporophylls was removed and discarded. The sporangia were then sterilized in 1.31% aqueous sodium hypochlorite solution and rinsed thoroughly with sterile, charcoal-distilled water in a germ-free transfer case in a P1 physical containment laboratory to insure that the culture plates were not contaminated with fungal spores. Both micro- and megaspores were inoculated on each plate using sterilized needles and forceps. The plates were sealed with parafilm to help retain moisture. Initially, the plates were kept in the laboratory where they were exposed to normal ambient light fluctuations. After about a month in which no prothallia were observed, the cultures were placed in darkness at a temperature of 0^0C for 2 days. Following removal from these conditions, megagametophytes at various stages of development were observed in 7–10 days. The mature female gametophyte of *I. engelmannii* exhibits an abundance of rhizoids, as well as a conspicuous protrusion of gametophytic tissue.

This technique should be suitable for other species of *Isoëtes* with little or no modification. Appreciation is extended to Bryan J. Taylor, Chief Park Naturalist of the North Carolina Division of Parks and Recreation Department, for granting a plant collecting permit.—*Sterling J. Sam, Department of Botany, Duke University, Durham, NC 27706.*